

CLAIMS

1. (originally presented) An *in vitro* method of determining activation or inactivation of the atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) hormonal systems, the method comprising simultaneously detecting the presence or amount of atrial and brain natriuretic peptide prohormones (proANP and proBNP) or fragments thereof in a sample.

2. (originally presented) A method according to claim 1, which comprises contacting the sample with a bi- or oligo- specific first binding substance that is able to bind to both:

- (a) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);
- (ii) a homologous sequence having at least 70% identity to (i); or
- (iii) a fragment of (i) or (ii) which is at least 6 amino acids in length;

and

- (b) (i) pro-BNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5) or NT-proBNP (SEQ ID NO. 6);
- (ii) a homologous sequence having at least 70% identity to (i); or
- (iii) a fragment of (i) or (ii) which is at least 6 amino acids in length.

3. (presently amended) A method according to claim 1 which comprises contacting the sample with

- an agent comprising:

- (a) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);
- (ii) a homologous sequence having at least 70% identity to (i); or
- (iii) a fragment of (i) or (ii) which is at least 6 amino acids in length;

and

- (b) (i) pro-BNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5) or NT-proBNP (SEQ ID NO. 6);

BEST AVAILABLE COPY

- (ii) a homologous sequence having at least 70% identity to (i); or
- ~~(iv)~~(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length.

and

- a first binding substance which is able to bind to:

- (a) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);
- (ii) a homologous sequence having at least 70% identity to (i); or
- (iii) a fragment of (i) or (ii) which is at least 6 amino acids in length;

and

- (b) (i) pro-BNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5) or NT-proBNP (SEQ ID NO. 6);
- (ii) a homologous sequence having at least 70% identity to (i); or
- ~~(iv)~~(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length;

and

- (c) the agent.

4. (originally presented) A method according to claim 3 wherein the first binding substance comprises:

- (a) a bi- or oligo-specific binding substance; or
- (b) a mixture of mono-specific binding substances.

5. (presently amended) A method according to ~~any one of claims 2 to 4~~ claim 2 wherein the first binding substance comprises:

- (a) natriuretic receptor GC-A (SEQ ID NO: 33);
- (b) homologous sequence having at least 70% identity to (a); or
- (c) a fragment of (a) or (b) which is at least 400 amino acids in length.

6. (originally presented) A method according to claim 5 wherein the first binding substance comprises an extracellular binding domain of the natriuretic receptor GC-A (SEQ ID NO: 34).

BEST AVAILABLE COPY

7. (presently amended) A method according to ~~any one of claims 2 to 4~~ claim 2 wherein the first binding substance comprises an antibody or a fragment or derivative thereof.

8. (originally presented) A method according to claim 7 wherein the antibody comprises a polyclonal antibody, monoclonal antibody, oligoclonal antibody, bifunctional antibody or crossreacting polyclonal antibody.

9. (presently amended) A method according to ~~any one of claims 3 to 8~~ claim 3 wherein in the agent, (a)(i) is SEQ ID NO. 3 and (b)(i) is SEQ ID NO: 6 or (a)(i) is SEQ ID NO. 2 and (b)(i) is SEQ ID NO. 5.

10. (presently amended) A method according to ~~any one of claims 3 to 8~~ claim 3 wherein the agent comprises or consists of:

- (a) proBNP₁₅₋₂₄ and proANP₂₁₋₉₆;
- (b) proBNP₁₋₃₇ and proANP₂₉₋₉₈;
- (c) proBNP₁₀₋₂₉ and proANP₂₀₋₈₀;
- (d) proBNP₁₋₇₆ and proANP₁₋₉₈;
- (e) proBNP₁₀₋₂₉ and proANP₆₀₋₈₀;
- (f) proBNP₁₋₁₀₈ and proANP₁₋₁₂₆; or
- (g) proBNP₇₇₋₉₂ and proANP₁₁₂₋₁₂₆.

11. (presently amended) A method according to ~~any one of claims 3 to 10~~ claim 3 wherein the agent is a polypeptide.

12. (presently amended) A method according to ~~any one of claims 2 to 11~~ claim 2 wherein the first binding substance and/or the agent is:

- (a) labelled with a detectable label; and/or
- (b) immobilised.

13. (presently amended) A method according to ~~any one of claims 2 to 12~~ claim 2 which additionally comprises contacting the sample with a second binding substance which is able to bind to the first binding substance.

14. (originally presented) A method according to claim 13 wherein the second binding substance is:

- (a) labelled with a detectable label; and/or
- (b) immobilised.

15. (originally presented) A method according to claim 13 wherein the second binding substance causes precipitation of the first binding substance and any peptide which is bound to it.

16. (presently amended) A method according to ~~any one of the preceding claims~~ claim 1 which comprises an immunoassay.

17. (presently amended) A method according to ~~any one of the preceding claims~~ claim 1 thereby to diagnose heart failure or monitor treatment of a cardiac condition.

18. (originally presented) An agent which comprises:

- (a) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);
- (ii) a homologous sequence having at least 70% identity to (i); or
- (iii) a fragment of (i) or (ii) which is at least 6 amino acids in length;

and

- (b) (i) pro-BNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5), NT-proBNP (SEQ ID NO. 6);
- (ii) a homologous sequence having at least 70% identity to (i); or
- (iii) a fragment of (i) or (ii) which is at least 6 amino acids in length.

19. (originally presented) An agent according to claim 18 which comprises or consists of:

- (a) proBNP₁₅₋₂₄ and proANP₈₂₋₉₆;
- (b) proBNP₁₋₃₇ and proANP₂₉₋₉₈;
- (c) proBNP₁₀₋₂₉ and proANP₂₀₋₈₀;
- (d) proBNP₁₋₇₆ and proANP₁₋₉₈;
- (e) proBNP₁₀₋₂₉ and proANP₆₀₋₈₀;
- (f) proBNP₁₋₁₀₈ and proANP₁₋₁₂₆; or
- (g) proBNP₇₇₋₉₂ and proANP₁₁₂₋₁₂₆.

20. (originally presented) An agent according to claim 19 which comprises any one of SEQ ID NOs. 13, 14, 15, 17, 18, 19 or 20.

21. (presently amended) An agent according to ~~any one of claims 18 to 20~~ claim 18 which is labelled with a detectable label.

22. (presently amended) A polypeptide agent according to ~~any one of claims 18 to 21~~ claim 18.

23. (originally presented) A polynucleotide comprising sequence which encodes a polypeptide according to claim 22 or sequence which is complementary to the coding sequence.

24. (originally presented) A polynucleotide according to claim 23 which comprises:

- (a) (i) SEQ ID NOs. 7, 8 or 9;
- (ii) a sequence complementary to (i);
- (iii) a sequence which hybridises under stringent conditions to (i) or (ii);
- (iv) a sequence which is degenerate as a result of the genetic code to (i), (ii) or (iii);

(v) a sequence having at least 70% identity to any of the sequences in (i) to (iv); or

(ii) a fragment of any of the sequences in (i) to (v);

and

(b) (i) SEQ ID NOs. 10, 11 or 12;

(ii) a sequence complementary to (i);

(iii) a sequence which hybridises under stringent conditions to (i) or (ii);

(iv) a sequence which is degenerate as a result of the genetic code to (i), (ii)

or (iii);

(v) a sequence having at least 70% identity to any of the sequences in (i) to (iv); or

(iii) a fragment of any of the sequences in (i) to (v).

25. (presently amended) An expression vector comprising a polynucleotide according to claim 23 or 24.

26. (presently amended) A host cell comprising a polynucleotide according to claim 23 or 24 or an expression vector according to claim 25.

27. (presently amended) A process for producing a polypeptide according to claim 22 which process comprises:

~~(a) (i) cultivating a host cell according to claim 26 comprising a polynucleotide, or its complement, which encodes a polypeptide which comprises:~~

(a) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length;

and

(b) (i) pro-BNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5), NT-proBNP (SEQ ID NO. 6);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length.
under conditions to provide for expression of the polypeptide; and optionally

(II) recovering the expressed polypeptide.

28. (originally presented) A process for producing a polypeptide according to claim 22 which comprises chemical synthesis.

29. (originally presented) A method of identifying a substance that binds specifically to

- (a) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);
- (ii) a homologous sequence having at least 70% identity to (i); or
- (iii) a fragment of (i) or (ii) which is at least 6 amino acids in length

and

- (b) (i) pro-BNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5), NT-proBNP (SEQ ID NO. 6);
- (ii) a homologous sequence having at least 70% identity to (i); or
- (iii) a fragment of (i) or (ii) which is at least 6 amino acids in length

which method comprises:

(A) contacting a candidate substance with (a) and (b) under conditions which allow specific binding; and

(B) determining whether the candidate substance binds to (a) and (b).

30. (presently amended) A method according to claim 29 which comprises:

(A) contacting the candidate substance with an agent ~~according to any one of claims 17 to 21;~~ which comprises:

- (a) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);
- (ii) a homologous sequence having at least 70% identity to (i); or
- (iii) a fragment of (i) or (ii) which is at least 6 amino acids in length;

and

BEST AVAILABLE COPY

(b) (i) pro-BNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5) or NT-proBNP (SEQ ID NO. 6);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length; and

(B) determining whether the candidate substance binds to the agent.

31. (originally presented) A bi- or oligo- specific antibody, fragment or derivative thereof which is able to bind to both:

(a) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length;

and

(b) (i) pro-BNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5) or NT-proBNP (SEQ ID NO. 6);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length.

32. (originally presented) An antibody, fragment or derivative according to claim 31 which is labelled with a detectable label.

33. (presently amended) A process for making an antibody as defined in claim 31 ~~or 32~~ comprising culturing a cell that expresses the antibody and optionally purifying antibody from the cell.

34. (presently amended) A process according to claim 33 in which the cell is one which is obtainable by administering ~~a polypeptide according to claim 22 to a mammal, a polypeptide agent which comprises:~~

(a) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length;

and

(b) (i) pro-BNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5) or NT-proBNP (SEQ ID NO. 6);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length

extracting B cells from the mammal and selecting a cell from these based on the ability to express an antibody with the specificity of ~~the antibody of claim 31~~ such that it is able to bind both

(a) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length;

and

(b) (i) pro-BNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5) or NT-proBNP (SEQ ID NO. 6);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length

35. (originally presented) A process according to claim 33 in which the cell is recombinant for a polynucleotide which expresses the antibody.

36. (presently amended) A solid support comprising an antibody according to claim 31 ~~or 32~~.

37. (originally presented) A solid support according to claim 36 which is a particle, dipstick or microtitre plate.

38. (presently amended) An agent which comprises

BEST AVAILABLE COPY

(a) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length;

and

(b) (i) pro-BNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5), NT-proBNP (SEQ ID NO. 6);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length.

according to any one of claims 17 to 22, a polynucleotide or its complement which encodes a polypeptide which comprises said agent according to claim 23 or 24 or an ~~bi~~ or oligo specific antibody, fragment or derivative thereof according to claim 31 or 32 which is able to bind said agent for use in treatment of the human or animal body by diagnosis or monitoring of treatment.

~~39. (cancelled) Use of a first binding substance as defined in any one of claims 2 to 8, an agent according to any one of claims 18 to 22, a polynucleotide according to claim 23 or 24 or an antibody according to claim 31 to 32 for the manufacture of a reagent for diagnosis and/or monitoring treatment of heart failure.~~

40. (presently amended) A diagnostic kit comprising:

(a) a bi or oligo specific first binding substance as defined in claim 2 that is able to bind to both

(i) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length;

and

BEST AVAILABLE COPY

(II) (i) pro-BNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5), NT-proBNP (SEQ ID NO. 6);

(ii) a homologous sequence having at least 70% identity to (i); or

(iv) a fragment of (i) or (ii) which is at least 6 amino acids in length; or

(b) a first binding substance and an agent as defined in claim 3;
wherein optionally the binding substance and/or the agent is labelled.

41. (presently amended) A kit according to claim 40 wherein the first binding substance is as
defined in any one of claims 4 to 8 comprises

(a) bi- or oligo-specific binding substance;

(b) a mixture of mono-specific binding substances

(c) natriuretic receptor GC-A (SEQ ID NO: 33)

(d) homologous sequence having at least 70% identity to (c)

(e) a fragment of (c) or (d) which is at least 400 amino acids in length

(f) an extracellular binding domain of the natriuretic receptor GC-A (SEQ ID NO:

34)

and/or is present on a solid support according to claim 36 or 37 comprising a bi- or oligo-specific antibody, fragment or derivative thereof which is able to bind to both;

(a) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length;

and

(b) (i) pro-BNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5) or NT-proBNP (SEQ ID NO. 6);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length.

42. (presently amended) A kit according to claim 40 or 41 wherein the agent is as defined in claims 18 to 22 comprises

(a) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length;

and

(b) (i) pro-BNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5), NT-proBNP (SEQ ID NO. 6);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length.

43. (presently amended) Use of:

- a first binding substance as defined in any one of claims 2 to 8, or

- an agent according to any one of claims 18 to 22

- a polynucleotide or its complement that encodes said first binding substance or

agent

- a bi or oligo specific antibody according to claim 31 to 32 fragment or derivative thereof which is able to bind said first binding substance or agent

- a solid support according to claim 26 or 27 which comprises said antibody; or

- a kit according to any one of claims 40 to 42 comprising said bi or oligo specific first binding substance and said agent

wherein said first binding substance or agent comprises:

(a) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length;

and

(b) (i) pro-BNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5), NT-proBNP (SEQ ID NO. 6);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length

in a method for diagnosis and/or monitoring treatment of heart failure.

44. (originally presented) A method of diagnosing and/or monitoring treatment of heart failure in an individual comprising:

- (a) obtaining a biological sample from an individual;
- (b) determining the activation or inactivation of both the ANP and BNP hormonal systems in the individual by a method which comprises simultaneously detecting the presence or amount of proANP and proBNP or fragments thereof in the sample.

45. (cancelled) ~~Use according to claim 43 or a method according to claim 44 which comprises a method as defined in any one of claims 1 to 17.~~

BEST AVAILABLE COPY